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METHODS AND COMPOSITIONS FOR TREATING PLATELET-RELATED DISORDERS

Related Applications

This application claims priority to U.S. Provisional Patent Application filed September 21, 1999, entitled "METHODS AND COMPOSITIONS FOR TREATING PLATELET-RELATED DISORDERS", Serial No.60/154,929, the contents of which are incorporated by reference herein in their entirety.

Field of the Invention

The invention relates to methods and products for treatment and/or prevention of platelet related thrombotic and other vaso-occlusive disorders.

Background of the Invention

Conditions resulting from thrombotic or thromboembolic events are the leading causes of illness and death in adults in western civilization. A great deal of effort and monetary resources have been directed towards understanding the mechanisms involved in vascular occlusive diseases involving thrombotic and thromboembolic events. These efforts have yielded a number of promising therapeutic agents. Notwithstanding the effort and financial resources that have been invested, these conditions still account for the vast majority of illness and death in the adult populations of developed nations.

Platelets are an important cellular component of blood involved in hemostasis as well as thrombotic or thromboembolic events. Abnormally high platelet counts such as those that result from hematological proliferative disorders such as for example essential thrombocythemia have been recognized as an important risk factor in thrombus formation. Furthermore, it has long been accepted that aspirin, which is known to inhibit cyclooxygenase and thereby prevents production of thromboxane A₂ in platelets, lowers the incidence of a thrombotic or thromboembolic events. For platelets, therapeutic regimens thus far reported have as their aim an inhibition of platelet function (e.g., inhibition of platelet adhesion, aggregation or factor release. An alternative modality is the reduction in platelets count in patients with abnormally high levels in certain hematological malignancies to levels approximating normal levels. Therapeutic intervention for reducing platelet count to low normal or below normal levels in subjects without myeloproliferative disorders has not been

proposed primarily since normal platelet count has been thought to be critical to normal hemostasis.

Summary of the Invention

The invention in a broad aspect involves the surprising discovery that subjects,
5 including those with normal levels of circulating platelets, can unexpectedly derive medical benefit from a reduction in platelet count to below normal levels, without serious adverse consequences as a result of the platelet count reduction. The benefit may be proportional or correlative to the reduction in platelet count in a broad safety range. Thus in situations where it is desirable to inhibit a pathological condition or process mediated in part by normal levels
10 of circulating platelets, subjects can be treated to lower platelet count preferably to a below normal level, thereby inhibiting the development, progression or propagation of the condition or accelerating or enhancing its regression. The methods of the invention are also useful for reducing the incidence of abnormal vessel growth induced by the presence of platelets.

A method is provided for treating a subject to reduce the risk of developing an adverse
15 condition or to inhibit the progression and consequences of an adverse condition mediated at least in part by platelets. In some aspects, the subject is treated to reduce platelet count to low normal levels, while in other aspects the subject is treated to reduce platelet count to below normal levels. In one embodiment, the subject is treated with a pharmaceutical agent.

In one aspect, the invention provides a method for treating a subject to inhibit a vaso-
20 occlusive event. Inhibiting a vaso-occlusive event means to prevent the formation of a vaso-occlusive event, to reduce progression and consequences of an already established vaso-occlusive event or to induce regression of a vaso-occlusive event. The invention also provides other methods aimed at reducing morbidity or mortality of subjects from vaso-occlusive events such as but not limited to thrombotic events which may lead to total or
25 partial vessel blockage by thrombus, or arterial stenosis due to excessive cell proliferation.

The methods of the invention comprise administering to a subject in need of such treatment an agent that reduces platelet count in the subject. The agent is administered in an amount effective to reduce platelet count in the subject to at least a low normal level. Such reductions in platelet count will reduce morbidity and/or mortality and thereby provide patient
30 outcome benefit.

As used herein, a vaso-occlusive event includes a pathological partial occlusion (including a narrowing) or complete occlusion of a blood vessel, a stent or a vascular graft. A vaso-occlusive event intends to embrace thrombotic or thromboembolic events, and the

vascular occlusion disorders or conditions to which they give rise. Thus, a vaso-occlusive event is intended to embrace all vascular occlusive disorders resulting in partial or total vessel occlusion from thrombotic or thromboembolic events, except those that are related to high platelet count due to a hematological proliferative disorder. A thrombotic event as used
5 herein is meant to embrace both a local thrombotic event and a distal thrombotic event (e.g., a thromboembolic event such as for example an embolic stroke). A vaso-occlusive event also includes abnormal blood vessel growth induced by the presence of platelets and the factors they secrete. An example of this latter form of vaso-occlusive event is intimal hyperplasia which results in a narrowing of the blood vessels (i.e., reduction in the diameter of blood
10 vessels either locally or throughout an extended segment of the vessel) due to a hyperproliferation of cells of the intimal layer of the blood vessel wall.

Preferably, the subject is otherwise free of symptoms calling for treatment with the agent. In some embodiments, the subject is preferably free of symptoms associated with a hematological proliferative disorder such as for example myeloproliferative disease.
15 Preferably, the subject is a human subject, but is not so limited. In another embodiment, the subject is apparently healthy. In preferred embodiments, the subjects do not have abnormally elevated platelet levels (i.e., a platelet count that is higher than the normal range) that are caused by a hematological proliferative disorder. Thus, preferably, the subjects do not have a hematological proliferative disorder. In an important embodiment, the subject has a normal
20 platelet count prior to treatment. In some embodiments, the subject has a higher platelet count than the mean normal level but is still considered within the normal range. As an example, a subject with a platelet count of 450×10^3 platelets per μl is considered to be at the high end of the normal range and is intended to be treated by the methods of the invention. In some embodiments, the subject may have a platelet count above the usual range, but without any
25 underlying hematological proliferative disorder. In another embodiment, the subject is not a post-menopausal female.

In some aspects, the invention intends to treat subjects who are at risk of a vaso-occlusive event. These subjects may or may not have had a previous vaso-occlusive event. The invention embraces the treatment of subjects prior to a vaso-occlusive event, at a time of
30 a vaso-occlusive event and following a vaso-occlusive event. Thus, as used herein, the "treatment" of a subject is intended to embrace both prophylactic and therapeutic treatment, and can be used both to limit or to eliminate altogether the symptoms or the occurrence of a

vaso-occlusive event. In one embodiment, the subject may exhibit symptoms of a vaso-occlusive event.

The invention also intends to embrace the treatment of a subject that has an abnormally elevated risk of a vaso-occlusive event such as a thrombotic event. The subject
5 may have vascular disease. The vascular disease may be selected from the group consisting of arteriosclerosis, cardiovascular disease, cerebrovascular disease, renovascular disease, mesenteric vascular disease, pulmonary vascular disease, ocular vascular disease or peripheral vascular disease.

In another embodiment, the subject has had a primary vaso-occlusive event such as a
10 primary thrombotic event. The agent may be administered to a subject following a primary vaso-occlusive event. The method of the invention embraces treatment of a subject to reduce the risk of a secondary thrombotic event or to inhibit the propagation of an existing thrombotic event. The thrombotic event may be selected from the group consisting of arterial thrombosis, coronary thrombosis, heart valve thrombosis, coronary stenosis, stent thrombosis,
15 and graft thrombosis. The vaso-occlusive event also includes disorders or conditions that may arise from a thrombotic event or a thromboembolic event and in this regard a vaso-occlusive event includes but is not limited to myocardial infarction, stroke and transient ischemic attack. In an important embodiment the vaso-occlusive event is myocardial infarction. In one embodiment, the subject has had a myocardial infarction. A subject who has
20 hypercholesterolemia, hypertension or atherosclerosis also can be treated by the methods of the invention.

In yet another embodiment, the subject is one who will undergo an elective surgical procedure. The agent may be administered to such a subject prior to the elective surgical procedure. The method of the invention can also be directed towards a subject who has
25 undergone a surgical procedure. As used herein, a surgical procedure is meant to embrace those procedures that have been classically regarded as surgical procedures as well as interventional cardiology procedures such as arteriography, angiography, angioplasty and stenting. Thus, the surgical procedure, whether elective or not, can be selected from the group consisting of coronary angiography, coronary stent placement, coronary by-pass surgery,
30 carotid artery procedure, peripheral stent placement, vascular grafting, thrombectomy, peripheral vascular surgery, vascular surgery, organ transplant, artificial heart transplant, vascular angioplasty, vascular laser therapy, vascular replacement, prosthetic valve replacement and vascular stenting.

In a preferred embodiment, the agent is anagrelide. In one embodiment, the agent is a derivative of anagrelide. In important embodiments, the agent is not a 2-aryl benzo[b]thiophene. In other important embodiments, the agent is not raloxifene hydrochloride. However, the agent is not an MPL pathway inhibitory agent (i.e., the agent does not impact upon the signal transduction pathway involving thrombopoietin and the Mpl receptor).

The agent is administered in an amount effective to reduce platelet count, in the subject, preferably to at least low normal levels. In one embodiment, the agent is administered in an amount ranging from 30 $\mu\text{g/kg/day}$ to 150 $\mu\text{g/kg/day}$. In another embodiment, the agent is administered in an amount ranging from 1 $\mu\text{g/kg/day}$ to 150 $\mu\text{g/kg/day}$. In some embodiments, these latter ranges are preferred when the agent is anagrelide or an anagrelide derivative.

In one embodiment, the agent is administered in an amount effective to reduce the platelet count to at least low normal levels if the subject has a normal platelet count prior to treatment. In another embodiment, the agent is administered in an amount effective to reduce

In some embodiments, the agent is administered in an amount effective to reduce the platelet count to below normal levels if the subject has an above normal platelet count prior to treatment. In these latter embodiments, the subject may not have a hematological proliferative disorder, but is not so limited.

Depending upon the particular embodiment, the platelet count is reduced anywhere from at least 10% to 95% of pre-treatment levels. In particular embodiments, the platelet count is reduced by at least 90%, at least 80%, at least 70%, at least 60%, at least 50%, at least 40%, at least 30%, at least 20%, or at least 10%. In some important embodiments, the platelet count is reduced by more than 10%. In another embodiment, platelet count is reduced by more than 30% or by more than 40%.

In some embodiments that embrace the treatment of a human subject, platelet count is preferably reduced to below 200×10^3 platelets per μl , and in still others to below 150×10^3 platelets per μl . In still another embodiment, platelet count is reduced to below 100×10^3 platelets per μl of blood in a human subject. In embodiments in which the platelet count is reduced to a low normal level this is defined as 10% less than the mean normal platelet count. In other embodiments, the platelet count is reduced to below normal levels.

In yet another embodiment, the agent is administered in an amount effective to reduce the platelet count by at least 10% and to an amount above 200×10^3 platelets per μl . In other

embodiments, the agent is administered in an amount effective to reduce the platelet count by at least 10% and below 200×10^3 platelets per μl . In other embodiments, the agent is administered in an amount effective to reduce the platelet count by at least 20% and to below 200×10^3 platelets per μl .

5 The agent of the invention can be administered simultaneously or consecutively with another therapeutic compound such as an agent which would normally be indicated for the subject. Such agents include agents for treating vascular disease or vascular complications (i.e., complications resulting from such disease). In some important embodiments, the agent for treating vascular disease or vascular complications is an anti-thrombotic agent. The anti-
10 thrombotic agent may be selected from the group consisting of an anti-coagulant agent, a fibrinolytic agent and an inhibitor of platelet function, but is not so limited. Thus, in one embodiment, the agent is administered with an inhibitor of platelet function. The inhibitor of platelet function may be selected from the group consisting of aspirin, abciximab, clopidogrel and dipyridamole. In another embodiment, the agent may be administered with an anti-
15 coagulant agent. The anti-coagulant may be selected from the group consisting of glycosaminoglycans (e.g., heparins) and vitamin K antagonists. In a further embodiment, the agent is administered with a fibrinolytic agent, such as but not limited to one selected from the group consisting of plasminogen activators such as tissue plasminogen activator (TPA), streptokinase and urokinase, plasmin and plasminogen. Depending upon the embodiment, the
20 agent of the invention may be administered before, simultaneously with or following administration of the agent for treating vascular disease or vascular complications.

Other useful categories of such agents include but are not limited to anti-inflammatory agents, anti-thrombotic agents, anti-platelet agents, fibrinolytic agents, lipid reducing agents, direct thrombin inhibitors, glycoprotein IIb/IIIa receptor inhibitors, agents that binds to
25 cellular adhesion molecules and inhibit the ability of white blood cells to attach to such molecules, calcium channel blockers, beta-adrenergic receptor blockers, cyclooxygenase-2 inhibitors, and angiotensin system inhibitors.

In one embodiment, the agent is administered following a primary vaso-occlusive event such as a thrombotic event. The agent can be administered in a number of ways,
30 including enteral and parenteral routes. In some preferred embodiments, the agent is administered in a sustained release device.

The invention also provides a number of pharmaceutical preparations comprising agents that reduce platelet count. The pharmaceutical preparations of the invention comprise

one or more agents that reduce platelet count and a pharmaceutically acceptable carrier. The agent is present in the pharmaceutical preparation in an amount effective to reduce platelet count. In important embodiments of the invention, the pharmaceutical preparation comprises the agent in an amount effective to reduce platelet count to low normal levels or to below normal levels.

In yet a further aspect, the invention provides a sustained release device that comprises an agent that reduces platelet count in a subject, wherein the agent is released for at least 7 days. In one embodiment, the sustained release device further comprises an agent for treating vascular disease or vascular complications. The agent for treating vascular disease or vascular complications may be an anti-thrombotic agent but is not so limited. In one embodiment, the anti-thrombotic is selected from the group consisting of an anti-coagulant agent, a fibrinolytic agent and an inhibitor of platelet function.

Preferably, the agent is released from the sustained release device in an amount effective to reduce platelet count in a subject to low normal or below normal levels. In one embodiment the agent is anagrelide or a derivative of anagrelide. Depending upon the embodiment and the nature of the agent, the sustained release device may release the agent at a rate ranging from 30 $\mu\text{g/kg/day}$ to 150 $\mu\text{g/kg/day}$. In other embodiments, the agent may be released at a rate ranging from 1 $\mu\text{g/kg/day}$ to 150 $\mu\text{g/kg/day}$. In one embodiment, the sustained release device releases the agent for at least 30 days. In other embodiments, the agent is released for at least 6 months, for at least 1 year, for at least 2 years or for at least 5 years or more. Preferably, the agent is released in an effective amount that does not affect platelet function.

In another aspect, a subject's blood is treated extracorporeally to reduce platelet count to low normal or below normal levels, using procedures such as by pheresis or adsorption of platelets and removal. Subjects, target platelet count and concurrent therapies are as described above. This aspect of the invention is particularly suited to acute therapy, although it is not so limited.

Detailed Description of the Invention

Unexpectedly, the invention involves the discovery that treating human subjects, especially those who do not have a hematological proliferative disorder, to induce a lower platelet count (such as to low normal levels or in some preferred instances to below normal levels) can have desirable medical benefit without significant adverse side effects.

The invention is premised in part on the discovery that a reduction in platelet count in a subject, such as for example to low normal and more preferably below normal levels, reduces the risk of a vaso-occlusive event such as a thrombotic event in the subject without significant adverse side effects. As used herein, a vaso-occlusive event is a event that is characterized by or results in a decrease in the internal diameter of blood vessels either locally or systemically to an extent which impedes blood flow in a subject and which for the purposes of the invention is of a pathological nature. Thus, a vaso-occlusive event embraces pathological narrowing or occlusion of a stent, a vascular graft or a blood vessel. As used herein, "pathological narrowing or occlusion" refers to narrowing or occlusion which is abnormal and/or disease-related. A vaso-occlusive event includes events which cause blood vessel narrowing or occlusion (such as thrombotic events, thromboembolic events and intimal hyperplasia) as well as conditions which result from such blood vessel narrowing (such as myocardial infarction and ischemic stroke).

A thrombotic event is an event associated with the formation or presence of a thrombus in a subject, particularly when present in the vasculature. A thrombus is an aggregation of blood factors, primarily platelets and fibrin with entrapment of cellular elements, frequently causing vascular obstruction at the point of its formation. Thrombotic events embrace thrombosis at a primary site as well as at a distal site (i.e., thromboembolism). Thrombosis collectively refers to diseases caused by the formation, development, or presence of a thrombus. Thromboembolism refers to diseases characterized by the blocking of a vessel, other than at the initial site of thrombus formation, by a thrombus which has been carried to the distal site by the blood current. As used herein, the term thrombosis is intended to embrace thromboembolism.

Thrombotic events including thromboembolic events can be serious medical conditions particularly since they can cause a reduction in blood flow to critical organs including the brain and myocardium. Examples of thrombotic events include but are not limited to arterial thrombosis, including stent and graft thrombosis, cardiac thrombosis, coronary thrombosis, heart valve thrombosis and venous thrombosis. Cardiac thrombosis is thrombosis in the heart. Arterial thrombosis is thrombosis in an artery. Coronary thrombosis is the development of an obstructive thrombus in a coronary artery, often causing sudden death or a myocardial infarction. Venous thrombosis is thrombosis in a vein. Heart valve thrombosis is a thrombosis on a heart valve. Stent thrombosis is thrombosis resulting from

and/or located in the vicinity of a vascular stent. Graft thrombosis is thrombosis resulting from and/or located in the vicinity of an implanted graft, particularly a vascular graft.

Examples of conditions or disorders that result from thrombotic events include but are not limited to myocardial infarction, stroke, transient ischemic attacks, amaurosis fugax, 5 aortic stenosis, cardiac stenosis, coronary stenosis and pulmonary stenosis. Stenosis is the narrowing or stricture of a duct or canal. Coronary stenosis is the narrowing or stricture of a coronary artery. Cardiac stenosis is a narrowing or diminution of any heart passage or cavity. Pulmonary stenosis is the narrowing of the opening between the pulmonary artery and the right ventricle. Aortic stenosis is narrowing of the aortic orifice of the heart or of the aorta 10 itself.

Vaso-occlusive events also include disorders in which the blood vessel narrowing results not necessarily from a thrombus but rather a thickening of the vessel wall such as with intimal hyperplasia. Intimal hyperplasia refers to a condition characterized by abnormal proliferation of the cells of the intimal layer of the blood vessel wall.

15 Thus, one aspect of the invention relates to a method for reducing the risk of a thrombotic event. In a particular embodiment, the method reduces the risk of stroke. Stroke is a condition resulting from the lack of oxygen to the brain, resulting from one or more occlusive thrombi. Depending on the area of the brain affected, stroke can result in a wide range of symptoms from transient ischemic attacks to death (e.g., coma, reversible or 20 irreversible paralysis, speech problems or dementia). In preferred embodiments, the stroke is non-hemorrhagic in nature.

The method of the invention in another embodiment relates to reducing the risk of myocardial infarction. Myocardial infarction refers to an irreversible injury to the heart muscle. Myocardial infarction generally results from an abrupt decrease in coronary blood 25 flow following a thrombotic occlusion (e.g., a thromboembolism) of a coronary artery. The thrombus, in many instances, forms after the rupture of atherosclerotic plaques in diseased coronary arteries. Such injury is highly correlated with factors such as cigarette smoking, hypertension and lipid accumulation.

Transient ischemic attack is a transient acute neurological dysfunction resulting from a 30 thromboembolism in the cerebral circulation. Amaurosis fugax is the temporary monocular blindness resulting from a thromboembolism in the retinal vasculature.

The methods of the invention can be used to reduce the risk of a primary or a secondary vaso-occlusive event such as a thrombotic event or to inhibit the progression of

such an event. A primary vaso-occlusive event refers to the first known vaso-occlusive event experienced by the subject. A secondary vaso-occlusive event refers to a vaso-occlusive event which occurs in a subject known or diagnosed as having previously experienced a vaso-occlusive event (i.e., a primary vaso-occlusive event).

5 According to the invention, the risk of a vaso-occlusive event such as a thrombotic event is reduced by administering to a subject an agent that reduces platelet count to a "low normal level" and in some embodiments to a "below normal level". A "normal" platelet count as used herein may be a level in a control population, which preferably includes subjects having similar characteristics as the treated individual, such as age and sex. The
10 "normal" level can also be a range, for example, where a population is used to obtain a baseline range for a particular group into which the subject falls. Thus, the "normal" value can depend upon a particular population selected. Preferably, the normal levels are those of apparently healthy subjects who have no prior history of platelet-mediated disorders. Such "normal" levels, then can be established as preselected values, taking into account the
15 category in which an individual falls. Appropriate ranges and categories can be selected with no more than routine experimentation by those of ordinary skill in the art. Either the mean or another preselected number within the range can be established as the normal preselected value.

As used herein, the terms "platelet level", "platelet number" and "platelet count" are
20 used interchangeably to refer to the number of platelets per a given volume of blood in a subject. The platelet count may be referred to in a number of ways (e.g., per μl of blood, per ml of blood, etc.). Generally, platelet counts are referred to herein as the number of platelets per μl of blood (i.e., platelets per μl), however other units may be used.

As is known in the art, the typical range for platelets in a "healthy" human subject is
25 about 150×10^3 to 450×10^3 platelets per μl of blood (mean 300×10^3 platelets per μl). Thus, "below normal levels" of platelets, as used herein, in this population is typically less than 150×10^3 platelets/ μl . "Low normal levels" as used herein refer to a platelet count which is 10% less than the mean normal platelet count. Thus, for the population just mentioned, low normal levels would be 270×10^3 platelets/ μl . Human subjects who have a platelet count of less than
30 100×10^3 platelets/ μl are considered thrombocytopenic. Platelet counts of less than 25×10^3 platelets/ μl indicate severe thrombocytopenia. The invention intends to embrace reductions in platelet counts resulting in platelet counts of equal to or less than 270×10^3 , 260×10^3 , 250×10^3 , 240×10^3 , 230×10^3 , 220×10^3 , 210×10^3 platelets/ μl of blood. In preferred

embodiments the platelet counts are equal to or less than 200×10^3 , 190×10^3 , 180×10^3 , 170×10^3 , 160×10^3 , 150×10^3 , 140×10^3 , 130×10^3 , 120×10^3 , 110×10^3 , 100×10^3 platelets/ μ l, above the level that is considered thrombocytopenic. Nonetheless, it will be understood that it may be desirable depending on factors such as the particular disease, and the age, physical
5 condition of the subject that lower levels are desirable, such as platelet counts equal to or less than 90×10^3 , 80×10^3 , 70×10^3 , 60×10^3 , 50×10^3 , and 25×10^3 platelets/ μ l of blood. In preferred embodiments, platelet count is reduced to below 200×10^3 platelets/ μ l in a human subject. In more preferred embodiments, the platelet count is reduced to below 150×10^3 platelets/ μ l while in some other even more preferred embodiments the platelet count is
10 reduced to below 100×10^3 platelets/ μ l in a human subject.

In some instances, it may be desirable to treat subjects having a platelet count in the normal range in order to reduce their platelet count and thereby reduce the risk of a vaso-occlusive event even if the post-treatment platelet count is still in the normal range. As an example, the methods of the invention may be used to treat a subject who has a platelet count
15 of 450×10^3 platelets/ μ l which while high, is still in the normal range. The subject may be treated in order to reduce the platelet count to either a lower level within the normal range (e.g., a low normal level, as described herein) or to a below normal level.

Platelet reductions may also be measured as a percentage of the pre-treatment platelet count in a subject. Thus the agents of the invention may be administered in an amount
20 effective to reduce platelet count from at least 10% to at least 95% of pre-treatment levels. In some embodiments, the agents are administered in an amount effective to reduce platelet count by at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% of pre-treatment levels. In some embodiments, the subjects are normal subjects who do not have an abnormally high level of circulating platelets such as a
25 platelet count greater than 500×10^3 platelets per μ l, or greater than 600×10^3 platelets per μ l which may be due to a hematological proliferative disorder. However, in other embodiments the subjects are normal subjects who have a high level of circulating platelets which is still however within the normal range. The invention intends to treat this latter group of subjects provided the subject does not have a hematological proliferative disorder such as
30 myeloproliferative disease. In still other embodiments, the subject may have a platelet count above the normal range, yet not have a hematological proliferative disorder. In preferred embodiments, platelets are reduced by at least 20% of pre-treatment levels. In more preferred

embodiments, platelets are reduced by at least 20% to at least 90% of pre-treatment levels. In still other embodiments, platelets are reduced by over 50% of pre-treatment levels.

The subjects may be treated so as to achieve both a drop in platelet count below an absolute level (such as for example below 200×10^3 platelets per μl) and a particular
5 percentage drop in platelet count relative to pre-treatment levels (such as for example at least 10%). As an example, a subject may be treated so as to reduce platelet count by at least 20% and to achieve a platelet count of less than 200×10^3 platelets per μl .

The invention intends to treat subjects who would benefit from inhibiting the growth of an existing thrombus or lowering of the risk of a vaso-occlusive event such as a thrombotic
10 event. A subject is a mammal including humans, nonhuman primates, dogs, cats, sheep, goats, horses, cows, pigs or rodents. The preferred subject is a human. The subject may be apparently healthy. An apparently healthy subject is one who, at the time of treatment, does not exhibit disease symptoms. In other words, such individuals, if examined by a medical professional, would be characterized as healthy and free of symptoms of disease. The
15 apparently healthy subjects however may still demonstrate particular risk factors which may place them at an elevated risk of a thrombotic event. For example, such subjects may be apparently healthy and still have a family history of thrombosis-related disorders. Alternatively, the subject may have symptoms of vaso-occlusive disease (such as chest pain, heart palpitations, shortness of breath, as well as a wide range of other symptoms well known
20 to a medical practitioner of ordinary skill) or may have been diagnosed with such disease.

In still other embodiments the subject is one who is otherwise free of symptoms calling for treatment with an agent that reduces, in the subject, platelet count. These subjects may not necessarily be apparently healthy but at a minimum they do not exhibit symptoms which ordinarily call for treatment specifically with an agent which reduces platelet count. As
25 an example, if the agent is anagrelide, the subject can be otherwise free of signs, symptoms or evidence of disorders for which anagrelide would normally be prescribed (e.g., myeloproliferative disease). Anagrelide has been prescribed previously for patients diagnosed with essential thrombocytosis (ET). The hallmark symptom of ET is an abnormally high level of platelets in the circulation, averaging about 600×10^3 platelets per μl of blood.
30 Involvement and expansion of other hemopoietic cell types is not necessarily a common feature of ET. Splenomegaly may also be observed in such patients. Secondary thrombocytosis is another disease state associated with an abnormally elevated number of platelets. This latter condition is distinguished from ET in that it results from a variety of

primary conditions such as recovery from acute infection, malignant diseases including carcinoma and lymphoma, hemolytic anemia, acute hemorrhage, iron deficiency, response to certain drugs and chronic inflammatory disorders. Subjects for whom the methods of the invention are not intended are those diagnosed with conditions which already call for
5 treatment with an agent such as anagrelide, i.e., secondary thrombocytosis, essential thrombocytosis, polycythemia vera, chronic myelogenous leukemia and myelofibrosis. In other words, in some preferred embodiments, the subject is not one who has, or who has been diagnosed with, a hematological proliferative disorder (such as myeloproliferative disease) which indicates the need for platelet lowering therapy.

10 The subject can also be one who is at abnormally elevated risk of a thrombotic event. The subject to be treated may be one who is prone to a thrombotic event. Included in this category of subjects are (1) those who have undergone a surgical procedure and are immobilized following such a procedure, (2) those who have chronic congestive heart failure, (3) those who have atherosclerotic vascular disease, (4) those who have malignancy
15 preferably other than a hematological malignancy which results in abnormally high platelet counts, and (5) those who are pregnant. A large majority of human subjects prone to thrombotic events do not manifest any observable perturbation in hemostasis.

One category of subjects with an abnormally elevated risk of a thrombotic event is those subjects who have previously experienced a primary thrombotic event. Subjects having
20 an abnormally elevated risk of a thrombotic event also include (i) those who have inherited a disposition towards thrombosis, for example those with a family history of thrombosis related disorders, (ii) those who have acquired a risk of a thrombotic event such as surgical patients, and (iii) those who engage in lifestyle habits which are considered high risk indicators for thrombosis.

25 One category of subjects with an abnormally elevated risk of thrombosis is those subjects having vascular disease. Vascular disease is a term which broadly encompasses all disorders of blood vessels (collectively known as the vasculature) including small and large arteries and veins, and blood flow. The most prevalent form of vascular disease is arteriosclerosis, a condition associated with the thickening and hardening of the arterial wall.
30 Arteriosclerosis or an arteriosclerotic condition as used herein means classical atherosclerosis, accelerated atherosclerosis, atherosclerosis lesions and any other arteriosclerotic conditions characterized by undesirable endothelial and/or vascular smooth muscle cell proliferation,

including vascular complications of diabetes. It is believed to be responsible for the majority of deaths in the United States and in most westernized societies.

Arteriosclerosis of the large vessels is referred to as atherosclerosis. Atherosclerosis is the predominant underlying factor in disorders such as coronary artery disease, aortic aneurysm, arterial disease of the lower extremities and cerebrovascular disease. Other types of arteriosclerosis include focal calcific arteriosclerosis (Mönckeberg's sclerosis) and arteriolosclerosis. Arterial diseases other than arteriosclerosis include congenital structural defects, inflammatory or granulomatous diseases (e.g., syphilitic aortitis), and small vessel disorders such as hypertension and autoimmune diseases. Disorders which are associated with early arteriosclerosis include diabetes mellitus, hypertension, familial hypercholesterolemia, familial combined hyperlipidemia, familial dysbetalipoproteinemia, familial hypoalphalipoproteinemia, hypothyroidism, cholesterol ester storage disease, systemic lupus erythematosus, homocysteinemia, chronic renal insufficiency, chronic vitamin D intoxication, pseudoxanthoma elasticum, idiopathic arterial calcification in infancy, aortic valvular calcification in the elderly and Werner's syndrome.

Subjects with cardiovascular disease, cerebrovascular disease and/or peripheral vascular disease (e.g., diabetic feet, failed grafts) are also considered at abnormally high risk of a thrombotic event. Cardiovascular disease refers to a number of disorders of the heart and vascular system. Cerebrovascular disease refers to a number of disorders of the blood vessels in the cerebrum or the brain. Peripheral vascular disease is disorder of the peripheral vasculature including that of the lower extremities.

The method of the invention can be used to treat subjects at abnormally elevated risk of experiencing particular vaso-occlusive events. For example, a subject with an abnormally elevated risk of myocardial infarction can be treated according to the method of the invention. Subjects may be treated prophylactically to reduce the risk of a primary or secondary myocardial infarction. As used herein, subjects having an abnormally elevated risk of myocardial infarction include those with unstable angina, multiple coronary risk factors, and Prinzmetal's variant angina. Less common etiologic factors include hypercoagulability, coronary emboli, collagen vascular disease, and cocaine abuse.

A subject with an abnormally elevated risk of stroke, for example non-hemorrhagic stroke, can also be treated according to the invention. Subjects having an abnormally elevated risk of an ischemic stroke are a category determined according to conventional medical practice; such subjects may also be identified in conventional medical practice as having

known risk factors for stroke or having increased risk of cerebrovascular events. The primary risk factors include hypertension, hypercholesterolemia, and smoking. Subjects having an abnormally elevated risk of an ischemic stroke also include individuals having any cardiac condition that may lead to decreased blood flow to the brain, such as atrial fibrillation, ventricular tachycardia, dilated cardiomyopathy and other cardiac conditions requiring anticoagulation. Subjects having an abnormally elevated risk of an ischemic stroke also include individuals having conditions including arteriopathy or brain vasculitis, such as that caused by lupus, congenital diseases of blood vessels, such as cadasil syndrome, or migraine, especially prolonged episodes.

Another category of subjects with an abnormally elevated risk of a thrombotic event are those subjects who will undergo or those who have already undergone a surgical or mechanical interventional procedure for the purposes of vessel repair and/or revascularization. Such procedures may be therapeutic or diagnostic in nature, and thus can also be elective or emergency treatments, and most likely involve the risk of formation of thrombi or the release of emboli. Procedures which fall into this category include but are not limited to vascular surgery including peripheral vascular surgery, vascular grafting, vascular laser therapy, vascular replacement, including prosthetic valve replacement, and vascular stenting, ventricular assist procedures, artificial heart transplant, heart and other organ transplants which require an interfacing of the transplanted organ with the vasculature of the transplant recipient, thrombectomy, coronary angiography, coronary and peripheral stent placements, carotid artery procedures including carotid endarterectomy, brain angiography, neurosurgical procedures in which blood vessels are compressed or occluded, cardiac catheterization, vascular angioplasty, including balloon angioplasty, coronary by-pass surgery. In addition to the risk of thrombus formation during or immediately following the surgical procedure, there also exists a risk to subjects who have undergone a surgical procedure and are currently immobilized following the procedure. Thus the invention seeks to embrace treatment of the subject prior to, during and following surgical procedures.

Other factors which predispose subjects to abnormally elevated risk of a thrombotic event are genetic risk factors and lifestyle habits. Inherited conditions can generally be regarded as hypercoaguable states or pre-thrombotic states. The pre-thrombotic subject can sometimes be identified if they present with a personal history of early (i.e., adolescent or as a young adult) and/or repeated thromboembolic events in the absence of an overt pre-disposing condition, and/or a family history of thrombosis related conditions. Subjects who have

experienced pain in walking, ischemia (i.e., a deficiency of blood flow to an area of the body due to functional constriction or obstruction of a blood vessel), gangrene (i.e., a death of tissue, usually considerable in mass and generally associated with loss of blood flow) and chest pain, may be regarded as having a personal history of arterial thrombosis or stroke, and are thus also at risk of a thrombotic event. Risk factors for a thrombotic event also include inheritable hematological abnormalities such as deficiency and/or dysfunction in any number of factors including anti-thrombin III, protein C, protein S and clotting factor V. Cardiovascular abnormalities, i.e., congenital structural abnormalities of the cardiovascular system, are also considered risk factors for thrombotic events. Vascular abnormalities such as atherosclerotic plaque ruptures are also considered a risk factor.

Lifestyle risk factors include smoking, failure to exercise and diet to the extent that it affects other risk factors such as obesity, high cholesterol, hyperlipidemia and high blood pressure (i.e., hypertension). High cholesterol (i.e., hypercholesterolemia), high blood pressure (i.e., hypertension), hyperlipidemia, and obesity are most certainly also induced by a variety of non-dietary causative elements including genetic and environmental factors.

A hyperlipidemic subject is defined as one whose cholesterol and triglyceride levels equal or exceed the limits set as described herein for both the hypercholesterolemic and hypertriglyceridemic subjects. A hypercholesterolemic subject (i.e., one with high cholesterol) has either an LDL (i.e., low-density lipoprotein) level of >160 mg/dL, or an LDL level of >130 mg/dL and at least two risk factors selected from the group consisting of male gender, family history of premature coronary heart disease, cigarette smoking (more than 10 cigarettes per day), hypertension, low HDL (<35 mg/dL), diabetes mellitus, hyperinsulinemia, abdominal obesity, high lipoprotein (a), and a personal history of cerebrovascular disease or occlusive peripheral vascular disease. A hypertriglyceridemic subject has a triglyceride (TG) level of >250 mg/dL.

Subjects who are hypertensive (i.e., those that have high blood pressure) are also at risk of a thrombotic event. A hypertensive subject is one who experiences persistently high arterial blood pressure. Hypertension may have no known cause, in which case it is referred to as essential or idiopathic hypertension. Alternatively, hypertension may be associated with other primary diseases, in which case it is referred to as secondary hypertension. It is generally considered a risk factor for the development of heart disease, peripheral vascular disease, stroke and kidney disease. In adults, a diastolic pressure below 85 mmHg is considered normal; between 85 and 89 mmHg is considered high normal, 90 to 104 mmHg is

considered mild hypertension, 105-114 mmHg is considered moderate hypertension and 115 mmHg or greater is considered severe hypertension. When the diastolic pressure is below 90 mmHg, a systolic pressure below 140 mmHg indicates normal blood pressure, between 140 and 159 mmHg is borderline isolated systolic hypertension and 160 mmHg or higher is
5 isolated systolic hypertension. Thus, generally, normal subjects are those with a blood pressure of 140/90 or less.

Other risk factors which contribute to an elevated risk of thrombotic events, and the disorders which underlie such thrombotic events (e.g., arteriosclerosis), include hyperlipidemia, hyperglycemia and diabetes mellitus, stress and personality, low index of
10 high density lipoproteins (HDL), male gender, age, hyperinsulinemia, high lipoprotein (a) and a personal history of cerebrovascular disease or occlusive peripheral vascular disease. Hyperglycemia is a condition associated with too high a level of glucose in the blood, sometimes indicative of uncontrolled diabetes. It occurs when the body does not have enough insulin or cannot effectively use insulin to metabolize glucose. This condition may be
15 associated with diabetes mellitus, Cushing's disease, and Cushing's syndrome. Signs of hyperglycemia are significant thirst, dry mouth, and frequent urination. Normal asymptomatic human subjects who are at least 50 years of age, and more preferably 60 years of age, are also at increased risk for thrombosis.

Subjects at risk of having intimal hyperplasia as well as those having intimal
20 hyperplasia are also intended to be treated according to the methods of the invention. Thus, the method of the invention can be used to treat subject who have or at risk of having intimal hyperplasia, as well as to reduce the risk of intimal hyperplasia. One common form of intimal hyperplasia is atherosclerosis.

The invention also intends to treat, in other aspects, subjects who have had a primary
25 vaso-occlusive event or who are currently experiencing a vaso-occlusive event, including subjects who have been diagnosed with thrombosis or as having a thrombotic event. The invention can also be used to treat subjects that have manifest an abnormal healing of blood vessels.

The treatment method of the invention involves the administration to a subject of an
30 agent that reduces circulating platelet count in the subject. Agents which reduce platelet count are herein sometimes referred to as platelet reducing agents. Preferably such agents have the specific effect of reducing only platelet count without affecting levels of other cell types, although it should be understood that an agent may also reduce levels of other cell

types provided these latter reductions do not induce unacceptable levels of adverse side effects associated with such reduction in other cell types. For example, the agent may reduce levels of megakaryocytes, the precursors of platelets, and such reduction should not have any undesirable side effect. As another example, an agent may be cytotoxic for a megakaryocyte lineage restricted cell, such as a platelet, and another blood cell, or a common precursor of these two cell types, in which case the agent is acceptable only if platelet count can be reduced to below normal levels without unacceptable levels of side effects associated with such reduction in the other cell type. In still another example, the agent may inhibit megakaryocyte function. It will be apparent to persons of ordinary skill in the art how to select and distinguish between such agents.

Agents already known to reduce platelet count include but are not limited to (1) cAMP phosphodiesterase inhibitors (e.g., anagrelide), 6,7-dichloro-1,5-dihydroimidazo-[2,1-b]quinazolin-2(3H)-one or 6,7-dichloro-1,2,3,5-tetrahydroimidazo[2,1-b]quinazolin-2-one (U.S. Patents 3,932,407; 4,146,718; RE31,617, Haematologica 1992 77:40-3), (2) antibodies to cell surface receptors specifically expressed by platelets or megakaryocytes such as glycoprotein IIb/IIIa receptor antibodies, (3) most chemotherapeutic anti-cancer drugs such as busulphan (Br. J. Haematol. 1986 62:229-37), hydroxyurea (N Engl J Med 1995 332:1132-6), hepsulfan, phosphorus-32 (Br J Radiol 1997 70:1169-73), pipobroman (Scand J. Haematol 1986 37:306-9), cyclophosphamide (J Cell Physiol 1982 112:222-8), certain alkylating agents and certain antimetabolites, (4) cytokines, growth factors and interleukins such as alpha-interferon (Cancer Immunol Immunother 1987 25:266-73), gamma-interferon, transforming growth factor-beta, neutrophil activating peptide-2 and its analogs (U.S. Patent 5,472,944), macrophage inflammatory protein and its analogs (U.S. Patent 5,306,709), (5) compounds secreted by either platelets or megakaryocytes such as platelet-factor 4 (U.S. Patent 5,185,323), transforming growth factor-beta, the 12-17 kD glycoprotein produced by megakaryocytes, thrombin and thrombospondin and its amino (1-174 amino acid) terminal fragment (J Lab Clin Med 1997 129:231-8), and (6) other agents including anti-cheloid agents such as Tranilast (Rizaben) (J Dermatol 1998 25:706-9); forskolin and spleen anti-maturation factor (U.S. Patent 4,088,753).

All the afore-mentioned agents may be suitable for use in the method of the invention to reduce normal platelet count in a subject preferably to a below normal level with the purpose of preventing or treating a vaso-occlusive event such as a thrombosis. In some instances these benefits are achieved by reducing the platelet count to low normal while in

other more preferred instances the platelet count is reduced to below normal levels. It should be understood that the agents useful in the invention may still be capable of affecting platelet function as well as reducing platelet count. However, preferably, such agents are used in a dose, formulation and administration schedule which favor the platelet count reducing activity of the agent and do not impact significantly, if at all, on platelet function.

Another category of agents which reduce platelet counts is MPL pathway inhibitory agents. However, the invention does not intend to embrace this category of agent in the methods provided herein.

A preferred agent is anagrelide. Although anagrelide is capable of affecting platelet function, it is used in the compositions and methods of the invention in a dose, formulation and administration schedule which reduces platelet count (preferably to below normal levels) without significantly impacting upon platelet function. Analogs (e.g., derivatives) of anagrelide which are as effective or more effective than the parent compound are also intended for use in the method of the invention. Preferably, such analogs would also be screened for an increased potency and specificity towards the megakaryocyte lineage with limited side effects. Synthesis of anagrelide analogs can be accomplished through routine chemical modification methods such as those routinely practiced in the art. Analogs of anagrelide have been reported by a number of groups. Jones et al. reported the synthesis of an analog, RS-82856 (J. Med. Chem. 1987 30:295-303). Other inhibitors of platelet cAMP phosphodiesterases synthesized by directed replacement of side chains on anagrelide have been reported by Meanwell et al. (J. Med. Chem. 1992 35:2672-87) Other anagrelide analogs have been documented in U.S. Patents 3,932,407; 4,146,718 and RE31,617.

Many of the above-listed agents while capable of reducing platelet count can also impact upon other cell lineages, particularly other hemopoietic cell lineages. It is preferred that the agents used in the methods of the invention are provided or administered in a manner which limits effects on other cell types. One way this can be accomplished is to identify agents which while perhaps not exclusive for the megakaryocyte lineage, have limited specificity for other cell lineages. Most of the agents listed above fall into this category. Another way of limiting unnecessary side effects is to administer the agent in the maximal dose which reduces platelet count and which does not impact upon other cell types. Such determination can be made using in vitro clonogenic assays such as those described herein, which are standard in the art. Yet another way of providing specificity is to conjugate an otherwise non-specific agent with a molecule which will target the non-specific agent to

megakaryocyte and platelets. Potential targeting molecules are those which normally bind receptors uniquely expressed on cells of the megakaryocyte lineage.

The methods of the invention utilize this library technology to identify small molecules including small peptides which bind to receptor ligand binding sites. One
5 advantage of using libraries for antagonist identification is the facile manipulation of millions of different putative candidates of small size in small reaction volumes (i.e., in synthesis and screening reactions). Another advantage of libraries is the ability to synthesize antagonists which might not otherwise be attainable using naturally occurring sources, particularly in the case of non-peptide moieties.

10 Methods for preparing libraries of molecules are well known in the art and many libraries are commercially available. Libraries of interest in the invention include synthetic organic combinatorial libraries. Libraries are also meant to include, but are not limited to, synthetic small molecule libraries and chemical libraries. The libraries can also comprise cyclic carbon or heterocyclic structure and/or aromatic or polyaromatic structures substituted
15 with one or more of the above-identified functional groups.

Small molecule combinatorial libraries may also be generated. A combinatorial library of small organic compounds is a collection of closely related analogs that differ from each other in one or more points of diversity and are synthesized by organic techniques using multi-step processes. Combinatorial libraries include a vast number of small organic
20 compounds. One type of combinatorial library is prepared by means of parallel synthesis methods to produce a compound array. A "compound array" as used herein is a collection of compounds identifiable by their spatial addresses in Cartesian coordinates and arranged such that each compound has a common molecular core and one or more variable structural diversity elements. The compounds in such a compound array are produced in parallel in
25 separate reaction vessels, with each compound identified and tracked by its spatial address. Examples of parallel synthesis mixtures and parallel synthesis methods are provided in U.S.S.N. 08/177,497, filed January 5, 1994 and its corresponding PCT published patent application W095/18972, published July 13, 1995 and U.S. Patent No. 5,712,171 granted January 27, 1998 and its corresponding PCT published patent application W096/22529, which
30 are hereby incorporated by reference.

One way of testing putatively useful agents is to perform in vitro assays in which platelets or platelet precursors (e.g., megakaryocytes, or megakaryocyte precursors) are exposed to a compound after which their morphology (for example using an appropriate cell

staining technique such as Wright's stain), number (for example using a Coulter counter) and/or colony forming ability are tested. These latter assays can be performed using either cell lines known to differentiate into the megakaryocyte lineage, or to of the megakaryocyte lineage, several of which have been established in the prior art and examples of which include the Ba/F3 and UT-7/GM cell line, or primary hemopoietic tissue, such as bone marrow. The number and quality of megakaryocyte colonies can be determined as a function of the presence and absence of the library member. Preferably, the assays are carried out by culturing the cells in a semi-solid culture in an amount of thrombopoietin sufficient to stimulate maximal megakaryocyte colony growth from the cell population. The library member is then titrated into the cultures in order to determine the amount necessary to reduce megakaryocyte colony formation. In this manner, in addition to the amount of antagonist necessary to inhibit megakaryocyte growth altogether, one can also determine that amount which inhibits the growth by a particular percentage. For example, if it desirable to reduce megakaryocyte growth and proliferation by 50% in order to achieve a reduction in platelet count *in vivo*, then the assay can be used to determine that amount of antagonist necessary to inhibit megakaryocyte colony growth by 50%. An important benefit of a clonogenic assay, such as that described herein, is the ability to analyze the effect of the library member on a wide variety of hemopoietic cell types. Since it is possible, with a correct cocktail of growth factors, to stimulate the growth of a variety of hemopoietic lineages in culture, the effect of the library member on each lineage can be studied. Thus, library members can be further screened for their selective action on the megakaryocyte lineage. Clonogenic assays such as those described herein are routinely employed by artisans of ordinary skill. Each of the aforementioned *in vitro* screening assays is amenable to high-throughput screening.

Another way of measuring the biological activity of the synthetic compound is to perform *in vivo* assays in which animals, preferably mice, are injected, for example intravenously, with the compound and then analyzed for megakaryocyte growth and proliferation or platelet production. Hemopoietic populations, such as bone marrow and spleen, can be harvested from treated animals and plated into *in vitro* semi-solid clonogenic cultures in order to determine the effect of the library member on megakaryocytes. Preferably the number and quality of megakaryocyte colonies derived from test animals should be compared to that of animals injected with control carrier (i.e., saline). Alternatively, animals can be assayed directly for platelet counts. This can be done in a number of ways including by bleeding the animals (usually from the tail or retro-orbital vein) and counting the number

of platelets either manually using a hemocytometer or through the use of an automated cell counter, such as a Coulter counter. Adverse side effects can also be tested in animals injected with putative antagonists in this manner. One possible adverse side effect may be an inability to clot due to a severe reduction in platelets. To assess clotting function, standard bleeding assays can be employed which measure the time required for bleeding from a experimentally induced wound to clot and thus stop. Platelet count and bleeding assays are routinely performed in human subjects as a measure of platelet count and platelet activity. Human subjects with a platelet count of more than 100×10^3 platelets per μl of blood are generally asymptomatic and their bleeding times are within the normal range. Bleeding times of less than 10 minutes are considered normal. When platelet count falls below 100×10^3 platelets per μl , the bleeding time is extended and appears to be linearly related to the platelet count. Human subjects with a platelet count of less than 50×10^3 platelets per μl experience easy bruising, while those with a platelet count of less than 20×10^3 platelets per μl are prone to spontaneous internal bleeding. Platelet count and bleeding assays are routinely practiced by those of ordinary skill in the art and are taught in Harrison's Principles of Internal Medicine, Isselbacher, McGraw Hill, New York (1994).

Physical methods also exist for reducing platelet count. These methods include platelet-pheresis which is the centrifugal separation of platelets from other blood cellular components. Platelet-pheresis provides the benefit of effecting platelet reduction in a short period of time. This may be desirable for a subject unexpectedly scheduled for an elective surgery. Other physical methods for reducing platelet count involve the use of adsorption of platelets onto solid state matrices coated with binding partners specific for platelets. As an example, platelets may be removed from blood using positive selection affinity filtration. Such an approach may involve applying peripheral blood to a column containing a solid matrix to which is coupled a growth factor such as thrombopoietin. Another example of affinity chromatography may involve elution of blood over an affinity matrix, having as the solid state Sephadex G-10, coated with fibrinogen, fibronectin, or preferably, an Arg-Gly-Asp tripeptide, all of which are known to bind platelets. This latter approach has been reported by Besselink et al. for binding of human platelets. (J. Biomater. Sci. Polym. Ed. 1995 7:551-562) Other binding partners for platelets which could be used in an adsorption technique to separate platelets from blood are glycoprotein IIb/IIIa antagonists such as Ro-43-8857 and L-700,462 reported by Cook et al. (Thromb. Haemost. 1993 70:838-47) Yet another form of affinity separation useful in the removal of platelets from blood is immune affinity which uses

a solid matrix coupled to an antibody specific for platelets and/or megakaryocytes such as an anti-glycoprotein IIb/IIIa receptor, or a fragment thereof.

Thus, in this aspect of the invention, a subject's blood is removed, depleted of platelets, and then returned until overall platelet count is below normal.

5 The invention provides pharmaceutical preparations of the agents of the invention. These pharmaceutical preparations comprise the agent of the invention and also a pharmaceutically acceptable carrier. The pharmaceutical preparations may be administered in effective amounts. The effective amount will depend upon the mode of administration, the particular condition being treated and the desired outcome. It will also depend upon, as
10 discussed above, the stage of the condition, the age and physical condition of the subject, the nature of concurrent therapy, if any, and like factors well known to the medical practitioner. For prophylactic applications, it is that amount sufficient to delay the onset of, inhibit the progression of, or halt altogether the particular condition being treated, thereby producing patient benefit. For therapeutic applications, it is that amount sufficient to achieve a
15 medically desirable result, thereby producing patient benefit. In some instances, patient benefit may be measured by a reduction in morbidity and/or mortality. In some cases this is a decrease in cell maturation and/or proliferation. In the case of megakaryocytes, the medically desirable result may be to inhibit thrombosis via blocking of megakaryocyte maturation, endoreduplication and/or proliferation. In other cases, it is an increase in platelet
20 consumption, elimination or death. Ultimately, the amount which is administered is one effective for reducing platelet count to low normal and more preferably below normal levels in a subject, without a significant level of adverse side effects.

 Generally, doses of active compounds of the present invention would be from about 0.01 mg/kg per day to 1000 mg/kg per day. It is expected that doses ranging from 1-500
25 mg/kg, and preferably doses ranging from 1-100 mg/kg, and even more preferably doses ranging from 1-50 mg/kg, will be suitable. In most preferred embodiments, the agents will be administered in doses ranging from 1 µg/kg/day to 10 mg/kg/day, with even more preferred doses ranging from 1 µg/kg/day to 150 µg/kg/day and from 30 µg/kg/day to 150 µg/kg/day depending upon the purpose of the intervention and the subject to be treated. The latter dose
30 range is preferred if the agent is anagrelide. A variety of administration routes are available. The methods of the invention, generally speaking, may be practiced using any mode of administration that is medically acceptable, meaning any enteral or parenteral mode that produces effective levels of the active compounds without causing clinically unacceptable

adverse effects. Such modes of administration include oral, rectal, topical, nasal, intrapulmonary, intracavitary, transdermal, interdermal, transmucosal, subcutaneous, intravenous, intraarterial, intramuscular, or local routes. The term "parenteral" includes subcutaneous, intravenous, intramuscular, or infusion. Injectable routes such as intravenous or intramuscular routes are not particularly suitable for long-term therapy and prophylaxis. They could, however, be preferred in situations where oral administration is contra-indicated. Oral administration will be preferred for prophylactic or therapeutic treatment because of the convenience to the patient as well as the dosing schedule.

Compositions suitable for oral administration may be presented as discrete units, in both immediate release or controlled release formulations such as capsules, tablets, lozenges, each containing a predetermined amount of the active agent. Other compositions include suspensions in aqueous liquids or non-aqueous liquids such as a syrup, elixir or an emulsion.

Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions as well as injectable drug delivery devices such as controlled release preparations. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like. Lower doses will result from other forms of administration, such as intravenous administration. In the event that a response in a subject is insufficient at the initial doses applied, higher doses (or effectively higher doses by a different, more localized delivery route) may be employed to the extent that patient tolerance permits. Multiple doses per day are contemplated to achieve appropriate systemic levels of compounds.

The agents that reduce platelet count may be combined, optionally, with a pharmaceutically-acceptable carrier. The term "pharmaceutically-acceptable carrier" as used herein means one or more compatible solid or liquid filler, diluents or encapsulating substances which are suitable for administration into a human. The term "carrier" denotes an organic or inorganic ingredient, natural or synthetic, with which the active ingredient is combined to facilitate the application. The components of the pharmaceutical compositions

also are capable of being co-mingled with the agents of the present invention, and with each other, in a manner such that there is no interaction which would substantially impair the desired pharmaceutical efficacy.

When administered, the pharmaceutical preparations of the invention are applied in
5 pharmaceutically-acceptable amounts and in pharmaceutically-acceptably compositions. Such preparations may routinely contain salt, buffering agents, preservatives, compatible carriers, and optionally other therapeutic agents. When used in medicine, the salts should be pharmaceutically acceptable, but non-pharmaceutically acceptable salts may conveniently be used to prepare pharmaceutically-acceptable salts thereof and are not excluded from the scope
10 of the invention. Such pharmacologically and pharmaceutically-acceptable salts include, but are not limited to, those prepared from the following acids: hydrochloric, hydrobromic, sulfuric, nitric, phosphoric, maleic, acetic, salicylic, citric, formic, malonic, succinic, and the like. Also, pharmaceutically-acceptable salts can be prepared as alkaline metal or alkaline earth salts, such as sodium, potassium or calcium salts.

15 Other delivery systems can include immediate release or controlled release formulations. Examples of controlled release formulations include time-release, delayed release or sustained release delivery systems. Such systems can reduce toxicity, increase efficacy and avoid repeated administrations of the platelet reducing agent, reducing peak-related side effects and increasing convenience to the subject and the physician. Many types
20 of release delivery systems are available and known to those of ordinary skill in the art. They include but are not limited to polymer base systems such as poly(lactide-glycolide), copolyoxalates, polycaprolactones, lipids, polyesteramides, polyorthoesters, polyhydroxybutyric acid, and polyanhydrides. Microcapsules of the foregoing polymers containing drugs are described in, for example, U.S. Patent 5,075,109 an non-polymer
25 systems such as melted and recrystallized sterols including cholesterol. Delivery systems also include non-polymer systems that are: lipids including sterols such as cholesterol, cholesterol esters and fatty acids or neutral fats such as mono- di- and tri-glycerides; hydrogel release systems; silastic systems; peptide based systems; wax coatings; compressed tablets using conventional binders and excipients; partially fused implants; and the like. Specific examples
30 include, but are not limited to: (a) erosional systems in which the platelet reducing agent is contained in a form within a matrix such as those described in U.S. Patent Nos. 4,452,775, 4,675,189 and 5,736,152 and (b) diffusional systems in which an active component permeates at a controlled rate from a polymer such as described in U.S. Patent Nos. 3,854,480,

5,133,974 and 5,407,686. In addition, pump-based hardware delivery systems can be used, some of which are adapted for implantation.

Use of a long-term sustained release implant (or device) may be particularly suitable for treatment of subjects at elevated risk of a vaso-occlusive event such as one resulting from a thrombotic event. These subjects would include subjects scheduled for elective vascular surgery. Long-term release, as used herein, means that the implant is constructed and arranged to deliver levels of the active ingredient for at least 1 week, in some instances for at least 30 days, and in others for at least 60 days. In some aspects of the invention that involve longer-term treatment and prevention, it is desirable that the sustained release device release effective amounts of agent for at least 6 months, 1 year, 2 years or in some cases, 5 years or more. Long-term sustained release implants are well-known to those of ordinary skill in the art and include some of the release systems described above.

Regardless of the particular agent used in the methods of the invention, administration via a sustained release device is preferable in some instances because it can reduce the peak levels of agent which are often observed with single bolus administrations (such as non-continuous injection or oral delivery). A reduction in the peak level of agent in the subject also reduces the likelihood of side effects. As an example, a side effect resulting from the ingestion of anagrelide is diarrhea. Sustained release of anagrelide would minimize this side effect.

The agent of the invention should be administered for a length of time sufficient to provide either or both therapeutic and prophylactic benefit to the subject. Generally, the agent is administered for at least one day. In some instances, particularly where a subject has had a vaso-occlusive event or where the subject is at risk of such an event, the agent may be administered for the remainder of the subject's life. The rate at which the agent is administered may vary depending upon the needs of the subject and the mode of administration. For example, it may be necessary in some instances to administer higher and more frequent doses of the agent to a subject for example during or immediately following a vaso-occlusive event (such as a myocardial infarction), provided still that such doses reduce platelet count but do not significantly affect platelet function. On the other hand, it may be desirable to administer lower doses in order to maintain a desired platelet count once it is achieved. In still other embodiments, the same dose of agent may be administered throughout the treatment period which as described herein may extend throughout the lifetime of the subject. The frequency of administration may vary depending upon the characteristics of the

subject. The agent may be administered daily, every 2 days, every 3 days, every 4 days, every 5 days, every week, every 10 days, every 2 weeks, every month, or more, or any time therebetween as if such time was explicitly recited herein.

In other aspects, the agents of the invention are administered with another agent, preferably an agent that would normally be indicated for the subject. In some embodiments, the agents may be administered substantially simultaneously with the other therapeutic agents. By substantially simultaneously, it is meant that a platelet reducing agent of the invention is administered to a subject close enough in time with the administration of the other therapeutic agent, whereby the two compounds may exert an additive or even synergistic effect, e.g., reducing platelet function by decreasing platelet count and inhibiting their ability to aggregate. In other embodiments, the platelet reducing agents of the invention be administered before or after the administration of the other therapeutic agent.

The agents of the invention may be administered with several categories of therapeutic agents, although preferably these agents are those which would normally be indicated for the subject. Generally, these agents are those which are useful and which are currently indicated for treating vascular disorders and vascular complications. These agents can be classified in terms of their function or in terms of the disorders for which they are indicated. Several useful categories of such agents include but are not limited to anti-inflammatory agents, anti-thrombotic agents, anti-platelet agents, fibrinolytic agents, lipid reducing agents, direct thrombin inhibitors, glycoprotein IIb/IIIa receptor inhibitors, agents that binds to cellular adhesion molecules and inhibit the ability of white blood cells to attach to such molecules, calcium channel blockers, beta-adrenergic receptor blockers, cyclooxygenase-2 inhibitors, and angiotensin system inhibitors.

One broad category agents which may be administered with the platelet reducing agents of the invention is anti-thrombotic agents. Anti-thrombotic agents are defined as agents which prevent the formation of a blood thrombus via a number of potential mechanisms and they include fibrinolytic agents, anti-coagulant agents and inhibitors of platelet function.

Fibrinolytic agents are defined as agent that lyse a thrombus (e.g., a blood clot), usually through the dissolution of fibrin by enzymatic action. Examples of thrombolytic agents include but are not limited to anecro, anistreplase, bisobrin lactate, brinolase, Hageman factor (i.e. factor XII) fragments, molsidomine, plasminogen activators such as streptokinase, tissue plasminogen activators (TPA) and urokinase, and plasmin and plasminogen. Anti-

coagulant agents also include inhibitors of factor Xa, factor TFPI, factor VIIa, factor IXc, factor Va, factor VIIIa as well as inhibitors of other coagulation factors.

Anti-coagulant agents are agents which inhibit the coagulation pathway by impacting negatively upon the production, deposition, cleavage and/or activation of factors essential in the formation of a blood clot. Anti-coagulant agents include but are not limited to vitamin K antagonists such as coumarin and coumarin derivatives (e.g., warfarin sodium); glycosoaminoglycans such as heparins both in unfractionated form and in low molecular weight form; ardeparin sodium, bivalirudin, bromindione, coumarin dalteparin sodium, desirudin, dicumarol, lyapolate sodium, nafamostat mesylate, phenprocoumon, sulfatide, and tinzaparin sodium.

Other "anti-coagulant" and/or "fibrinolytic" agents include Plasminogen (to plasmin via interactions of prekallikrein, kininogens, Factors XII, XIIIa, plasminogen proactivator, and tissue plasminogen activator[TPA]) Streptokinase; Urokinase; Anisoylated Plasminogen-Streptokinase Activator Complex; Pro-Urokinase; (Pro-UK); rTPA (alteplase or activase; r denotes recombinant); rPro-UK; Abbokinase; Eminase; Streptase Anagrelide Hydrochloride; Bivalirudin; Dalteparin Sodium; Danaparoid Sodium; Dazoxiben Hydrochloride; Efegatran Sulfate; Enoxaparin Sodium; Ifetroban; Ifetroban Sodium; Tinzaparin Sodium; retaplast; Trifenagrel; Warfarin; Dextran.

Still other anti-coagulant agents include, but are not limited to, Ancrod; Anticoagulant Citrate Dextrose Solution; Anticoagulant Citrate Phosphate Dextrose Adenine Solution; Anticoagulant Citrate Phosphate Dextrose Solution; Anticoagulant Heparin Solution; Anticoagulant Sodium Citrate Solution; Ardeparin Sodium; Bivalirudin; Bromindione; Dalteparin Sodium; Desirudin; Dicumarol; Heparin Calcium; Heparin Sodium; Lyapolate Sodium; Nafamostat Mesylate; Phenprocoumon; Tinzaparin Sodium; Warfarin Sodium.

Heparin may stabilize symptoms in evolving stroke, but anticoagulants are useless (and possibly dangerous) in acute completed stroke, and are contraindicated in hypertensives because of the increased possibility of hemorrhage into the brain or other organs. Although the timing is controversial, anticoagulants may be started to prevent recurrent cardiogenic emboli. Clot lysing agents, including tissue plasminogen activator and streptokinase, are being evaluated for the very early treatment of acute stroke. Nimodipine has recently been shown to improve survival and clinical outcome after ischemic stroke.

Inhibitors of platelet function are agents that impair the ability of mature platelets to perform their normal physiological roles (i.e., their normal function). Platelets are normally

involved in a number of physiological processes such as adhesion, for example, to cellular and non-cellular entities, aggregation, for example, for the purpose of forming a blood clot, and release of factors such as growth factors (e.g., platelet-derived growth factor (PDGF)) and platelet granular components. One subcategory of platelet function inhibitors are
5 inhibitors of platelet aggregation which are compounds which reduce or halt the ability of platelets to associate physically with themselves or with other cellular and non-cellular components, thereby precluding the ability of a platelet to form a thrombus.

Examples of useful inhibitors of platelet function include but are not limited to
10 acadesine, anagrelide (if given at doses exceeding 10 mg/day), anipamil, argatroban, aspirin, clopidogrel, cyclooxygenase inhibitors such as nonsteroidal anti-inflammatory drugs and the synthetic compound FR-122047, danaparoid sodium, dazoxiben hydrochloride, diadenosine 5',5'''-P₁,P₄-tetrphosphate (Ap₄A) analogs, difibrotide, dilazep dihydrochloride, 1,2- and 1,3-glyceryl dinitrate, dipyridamole, dopamine and 3-methoxytyramine, efegatran sulfate, enoxaparin sodium, glucagon, glycoprotein IIb/IIIa antagonists such as Ro-43-8857 and L-
15 700,462, ifetroban, ifetroban sodium, iloprost, isocarbacyclin methyl ester, isosorbide-5-mononitrate, itazigrel, ketanserin and BM-13.177, lamifiban, lifarizine, molsidomine, nifedipine, oxagrelate, PGE, platelet activating factor antagonists such as lexipafant, prostacyclin (PGI₂), pyrazines, pyridinol carbamate, ReoPro (i.e., abciximab), sulfinpyrazone, synthetic compounds BN-50727, BN-52021, CV-4151, E-5510, FK-409, GU-7, KB-2796,
20 KBT-3022, KC-404, KF-4939, OP-41483, TRK-100, TA-3090, TFC-612 and ZK-36374, 2,4,5,7-tetrathiaoctane, 2,4,5,7-tetrathiaoctane 2,2-dioxide, 2,4,5-trithiahexane, theophyllin pentoxifyllin, thromboxane and thromboxane synthetase inhibitors such as picotamide and sulotroban, ticlopidine, tirofiban, trapidil and ticlopidine, trifenagrel, trilinolein, 3-substituted 5,6-bis(4-methoxyphenyl)-1,2,4-triazines, and antibodies to glycoprotein IIb/IIIa as well as
25 those disclosed in U.S. Patent 5,440,020, and anti-serotonin drugs, Clopidogrel; Sulfinpyrazone; Aspirin; Dipyridamole; Clofibrate; Pyridinol Carbamate; PGE; Glucagon; Antiserotonin drugs; Caffeine; Theophyllin Pentoxifyllin; Ticlopidine.

"Anti-inflammatory" agents include Alclofenac; Alclometasone Dipropionate; Algestone Acetonide; Alpha Amylase; Amcinafal; Amcinafide; Amfenac Sodium; Amiprilose
30 Hydrochloride; Anakinra; Anirolac; Anitrazafen; Apazone; Balsalazide Disodium; Bendazac; Benoxaprofen; Benzydamine Hydrochloride; Bromelains; Broperamole; Budesonide; Carprofen; Cicloprofen; Cintazone; Cliprofen; Clobetasol Propionate; Clobetasone Butyrate; Clopirac; Cloticasone Propionate; Cormethasone Acetate; Cortodoxone; Deflazacort;

Desonide; Desoximetasone; Dexamethasone Dipropionate; Diclofenac Potassium; Diclofenac Sodium; Diflorasone Diacetate; Diflumidone Sodium; Diflunisal; Difluprednate; Diftalone; Dimethyl Sulfoxide; Drocinnonide; Endrysone; Enlimomab; Enolicam Sodium; Epirizole; Etodolac; Etofenamate; Felbinac; Fenamole; Fenbufen; Fenclofenac; Fenclorac; Fendosal; 5 Fenpivalone; Fentiazac; Flazalone; Fluazacort; Flufenamic Acid; Flumizole; Flunisolid Acetate; Flunixin; Flunixin Meglumine; Fluocortin Butyl; Fluorometholone Acetate; Fluquazone; Flurbiprofen; Fluretofen; Fluticasone Propionate; Furaprofen; Furobufen; Halcinonide; Halobetasol Propionate; Halopredone Acetate; Ibufenac; Ibuprofen; Ibuprofen Aluminum; Ibuprofen Piconol; Ilonidap; Indomethacin; Indomethacin Sodium; Indoprofen; 10 Indoxole; Intrazole; Isoflupredone Acetate; Isoxepac; Isoxicam; Ketoprofen; Lofemizole Hydrochloride; Lornoxicam; Loteprednol Etabonate; Meclofenamate Sodium; Meclofenamic Acid; Meclorisone Dibutyrate; Mefenamic Acid; Mesalamine; Meseclazone; Methylprednisolone Suleptanate; Morniflumate; Nabumetone; Naproxen; Naproxen Sodium; Naproxol; Nimazone; Olsalazine Sodium; Orgotein; Orpanoxin; Oxaprozin; 15 Oxyphenbutazone; Paranyline Hydrochloride; Pentosan Polysulfate Sodium; Phenbutazone Sodium Glycerate; Pirfenidone; Piroxicam; Piroxicam Cinnamate; Piroxicam Olamine; Pirprofen; Prednazate; Prifelone; Prodolic Acid; Proquazone; Proxazole; Proxazole Citrate; Rimexolone; Romazarit; Salcolex; Salnacedin; Salsalate; Salicylates; Sanguinarium Chloride; Seclazone; Sermetacin; Sudoxicam; Sulindac; Suprofen; Talmetacin; Talniflumate; 20 Talosalate; Tebufelone; Tenidap; Tenidap Sodium; Tenoxicam; Tesicam; Tesimide; Tetrydamine; Tiopinac; Tixocortol Pivalate; Tolmetin; Tolmetin Sodium; Triclonide; Triflumidate; Zidometacin; Glucocorticoids; Zomepirac Sodium. One preferred anti-inflammatory agent is aspirin.

“Lipid reducing” agents include gemfibrozil, cholestyramine, colestipol, nicotinic 25 acid, probucol lovastatin, fluvastatin, simvastatin, atorvastatin, pravastatin, cirivastatin.

“Direct thrombin inhibitors” include hirudin, hirugen, hirulog, agatroban, PPACK, thrombin aptamers.

“Glycoprotein IIb/IIIa receptor inhibitors” are both antibodies and non-antibodies, and include but are not limited to ReoPro (abciximab), lamifiban, tirofiban.

30 “Calcium channel blockers” are a chemically diverse class of compounds having important therapeutic value in the control of a variety of diseases including several cardiovascular disorders, such as hypertension, angina, and cardiac arrhythmias (Fleckenstein, *Cir. Res.* v. 52, (suppl. 1), p.13-16 (1983); Fleckenstein, *Experimental Facts and Therapeutic*

Prospects, John Wiley, New York (1983); McCall, D., *Curr Pract Cardiol*, v. 10, p. 1-11 (1985)). Calcium channel blockers are a heterogeneous group of drugs that prevent or slow the entry of calcium into cells by regulating cellular calcium channels. (Remington, *The Science and Practice of Pharmacy*, Nineteenth Edition, Mack Publishing Company, Eaton, PA, p.963 (1995)). Most of the currently available calcium channel blockers, and useful according to the present invention, belong to one of three major chemical groups of drugs, the dihydropyridines, such as nifedipine, the phenyl alkyl amines, such as verapamil, and the benzothiazepines, such as diltiazem. Other calcium channel blockers useful according to the invention, include, but are not limited to, amrinone, amlodipine, bencyclane, felodipine, fendiline, flunarizine, isradipine, nicardipine, nimodipine, perhexilene, gallopamil, tiapamil and tiapamil analogues (such as 1993RO-11-2933), phenytoin, barbiturates, and the peptides dynorphin, omega-conotoxin, and omega-agatoxin, and the like and/or pharmaceutically acceptable salts thereof.

"Beta-adrenergic receptor blocking agents" are a class of drugs that antagonize the cardiovascular effects of catecholamines in angina pectoris, hypertension, and cardiac arrhythmias. Beta-adrenergic receptor blockers include, but are not limited to, atenolol, acebutolol, alprenolol, befunolol, betaxolol, bunitrolol, carteolol, celiprolol, hedroxalol, indenolol, labetalol, levobunolol, mepindolol, methypranol, metindol, metoprolol, metrizoranolol, oxprenolol, pindolol, propranolol, practolol, practolol, sotalolnadolol, tiprenolol, tomalolol, timolol, bupranolol, penbutolol, trimepranol, 2-(3-(1,1-dimethylethyl)-amino-2-hydroxypropoxy)-3-pyridenecarbonitrilHCl, 1-butylamino-3-(2,5-dichlorophenoxy)-2-propanol, 1-isopropylamino-3-(4-(2-cyclopropylmethoxyethyl)phenoxy)-2-propanol, 3-isopropylamino-1-(7-methylindan-4-yloxy)-2-butanol, 2-(3-t-butylamino-2-hydroxy-propylthio)-4-(5-carbamoyl-2-thienyl)thiazol, 7-(2-hydroxy-3-t-butylaminpropoxy)phthalide. The above-identified compounds can be used as isomeric mixtures, or in their respective levorotating or dextrorotating form.

Cyclooxygenase-2 (COX-2) is a recently identified form of a cyclooxygenase. "Cyclooxygenase" is an enzyme complex present in most tissues that produces various prostaglandins and thromboxanes from arachidonic acid. Non-steroidal, anti-inflammatory drugs exert most of their anti-inflammatory, analgesic and antipyretic activity and inhibit hormone-induced uterine contractions and certain types of cancer growth through inhibition of the cyclooxygenase (also known as *prostaglandin G/H synthase* and/or *prostaglandin-endoperoxide synthase*). Initially, only one form of cyclooxygenase was known, the

“constitutive enzyme” or cyclooxygenase-1 (COX-1). It was originally identified in bovine seminal vesicles.

Cyclooxygenase-2 (COX-2) has been cloned, sequenced and characterized initially from chicken, murine and human sources (See, e.g., U.S. Patent 5,543,297, issued August 6, 1996 to Cromlish, *et al.*, and assigned to Merck Frosst Canada, Inc., Kirkland, CA, entitled: “Human cyclooxygenase-2 cDNA and assays for evaluating cyclooxygenase-2 activity”). This enzyme is distinct from the COX-1. COX-2 is rapidly and readily inducible by a number of agents including mitogens, endotoxin, hormones, cytokines and growth factors. As prostaglandins have both physiological and pathological roles, the constitutive enzyme, COX-1, is responsible, in large part, for endogenous basal release of prostaglandins and hence is important in their physiological functions such as the maintenance of gastrointestinal integrity and renal blood flow. By contrast, it is believed that the inducible form, COX-2, is mainly responsible for the pathological effects of prostaglandins where rapid induction of the enzyme would occur in response to such agents as inflammatory agents, hormones, growth factors, and cytokines. Therefore, it is believed that a selective inhibitor of COX-2 has similar anti-inflammatory, antipyretic and analgesic properties to a conventional non-steroidal anti-inflammatory drug, and in addition inhibits hormone-induced uterine contractions and also has potential anti-cancer effects, but with reduced side effects. In particular, such COX-2 inhibitors are believed to have a reduced potential for gastrointestinal toxicity, a reduced potential for renal side effects, a reduced effect on bleeding times and possibly a decreased potential to induce asthma attacks in aspirin-sensitive asthmatic subjects, and are therefore useful according to the present invention.

A number of selective “COX-2 inhibitors” are known in the art. These include, but are not limited to, COX-2 inhibitors described in U.S. Patent 5,474,995 “Phenyl heterocycles as cox-2 inhibitors”; U.S. Patent 5,521,213 “Diaryl bicyclic heterocycles as inhibitors of cyclooxygenase-2”; U.S. Patent 5,536,752 “Phenyl heterocycles as COX-2 inhibitors”; U.S. Patent 5,550,142 “Phenyl heterocycles as COX-2 inhibitors”; U.S. Patent 5,552,422 “Aryl substituted 5,5 fused aromatic nitrogen compounds as anti-inflammatory agents”; U.S. Patent 5,604,253 “N-benzylindol-3-yl propanoic acid derivatives as cyclooxygenase inhibitors”; U.S. Patent 5,604,260 “5-methanesulfonamido-1-indanones as an inhibitor of cyclooxygenase-2”; U.S. Patent 5,639,780 N-benzyl indol-3-yl butanoic acid derivatives as cyclooxygenase inhibitors”; U.S. Patent 5,677,318 Diphenyl-1,2,3-thiadiazoles as anti-inflammatory agents”; U.S. Patent 5,691,374 “Diaryl-5-oxygenated-2-(5H) -furanones as

COX-2 inhibitors"; U.S. Patent 5,698,584 "3,4-diaryl-2-hydroxy-2,5-dihydrofurans as prodrugs to COX-2 inhibitors"; U.S. Patent 5,710,140 "Phenyl heterocycles as COX-2 inhibitors"; U.S. Patent 5,733,909 "Diphenyl stilbenes as prodrugs to COX-2 inhibitors"; U.S. Patent 5,789,413 "Alkylated styrenes as prodrugs to COX-2 inhibitors"; U.S. Patent 5,817,700 "Bisaryl cyclobutenes derivatives as cyclooxygenase inhibitors"; U.S. Patent 5,849,943 "Stilbene derivatives useful as cyclooxygenase-2 inhibitors"; U.S. Patent 5,861,419 "Substituted pyridines as selective cyclooxygenase-2 inhibitors"; U.S. Patent 5,922,742 "Pyridinyl-2-cyclopenten-1-ones as selective cyclooxygenase-2 inhibitors"; U.S. Patent 5,925,631 "Alkylated styrenes as prodrugs to COX-2 inhibitors"; all of which are commonly assigned to Merck Frosst Canada, Inc. (Kirkland, CA). Additional COX-2 inhibitors are also described in U.S. Patent 5,643,933, assigned to G. D. Searle & Co. (Skokie, IL), entitled: "Substituted sulfonylphenylheterocycles as cyclooxygenase-2 and 5-lipoxygenase inhibitors."

A number of the above-identified COX-2 inhibitors are prodrugs of selective COX-2 inhibitors, and exert their action by conversion *in vivo* to the active and selective COX-2 inhibitors. The active and selective COX-2 inhibitors formed from the above-identified COX-2 inhibitor prodrugs are described in detail in WO 95/00501, published January 5, 1995, WO 95/18799, published July 13, 1995 and U.S. Patent 5,474,995, issued December 12, 1995. Given the teachings of U.S. Patent 5,543,297, entitled: "Human cyclooxygenase-2 cDNA and assays for evaluating cyclooxygenase-2 activity," a person of ordinary skill in the art would be able to determine whether an agent is a selective COX-2 inhibitor or a precursor of a COX-2 inhibitor, and therefore part of the present invention.

An "angiotensin system inhibitor" is an agent that interferes with the function, synthesis or catabolism of angiotensin II. These agents include, but are not limited to, angiotensin-converting enzyme (ACE) inhibitors, angiotensin II antagonists, angiotensin II receptor antagonists, agents that activate the catabolism of angiotensin II, and agents that prevent the synthesis of angiotensin I from which angiotensin II is ultimately derived. The renin-angiotensin system is involved in the regulation of hemodynamics and water and electrolyte balance. Factors that lower blood volume, renal perfusion pressure, or the concentration of Na⁺ in plasma tend to activate the system, while factors that increase these parameters tend to suppress its function.

Angiotensin I and angiotensin II are synthesized by the enzymatic renin-angiotensin pathway. The synthetic process is initiated when the enzyme renin acts on angiotensinogen, a

pseudoglobulin in blood plasma, to produce the decapeptide angiotensin I. Angiotensin I is converted by angiotensin converting enzyme (ACE) to angiotensin II (angiotensin-[1-8] octapeptide). The latter is an active pressor substance which has been implicated as a causative agent in several forms of hypertension in various mammalian species, e.g., humans.

5 Angiotensin (renin-angiotensin) system inhibitors are compounds that act to interfere with the production of angiotensin II from angiotensinogen or angiotensin I or interfere with the activity of angiotensin II. Such inhibitors are well known to those of ordinary skill in the art and include compounds that act to inhibit the enzymes involved in the ultimate production of angiotensin II, including renin and ACE. They also include compounds that interfere with
10 the activity of angiotensin II, once produced. Examples of classes of such compounds include antibodies (e.g., to renin), amino acids and analogs thereof (including those conjugated to larger molecules), peptides (including peptide analogs of angiotensin and angiotensin I), pro-renin related analogs, etc. Among the most potent and useful renin-angiotensin system inhibitors are renin inhibitors, ACE inhibitors, and angiotensin II antagonists. In a preferred
15 embodiment of the invention, the renin-angiotensin system inhibitors are renin inhibitors, ACE inhibitors, and angiotensin II antagonists.

 "Angiotensin II antagonists" are compounds which interfere with the activity of angiotensin II by binding to angiotensin II receptors and interfering with its activity. Angiotensin II antagonists are well known and include peptide compounds and non-peptide
20 compounds. Most angiotensin II antagonists are slightly modified congeners in which agonist activity is attenuated by replacement of phenylalanine in position 8 with some other amino acid; stability can be enhanced by other replacements that slow degeneration *in vivo*. Examples of angiotensin II antagonists include: peptidic compounds (e.g., saralasin, [(San¹)(Val⁵)(Ala⁸)] angiotensin -(1-8) octapeptide and related analogs); N-substituted
25 imidazole-2-one (US Patent Number 5,087,634); imidazole acetate derivatives including 2-N-butyl-4-chloro-1-(2-chlorobenzyl) imidazole-5-acetic acid (see Long et al., *J. Pharmacol. Exp. Ther.* 247(1), 1-7 (1988)); 4, 5, 6, 7-tetrahydro-1H-imidazo [4, 5-c] pyridine-6-carboxylic acid and analog derivatives (US Patent Number 4,816,463); N2-tetrazole beta-glucuronide analogs (US Patent Number 5,085,992); substituted pyrroles, pyrazoles, and
30 tryazoles (US Patent Number 5,081,127); phenol and heterocyclic derivatives such as 1, 3-imidazoles (US Patent Number 5,073,566); imidazo-fused 7-member ring heterocycles (US Patent Number 5,064,825); peptides (e.g., US Patent Number 4,772,684); antibodies to angiotensin II (e.g., US Patent Number 4,302,386); and aralkyl imidazole compounds such as

biphenyl-methyl substituted imidazoles (e.g., EP Number 253,310, January 20, 1988); ES8891 (N-morpholinoacetyl-(-1-naphthyl)-L-alanyl-(4, thiazolyl)-L-alanyl (35, 45)-4-amino-3-hydroxy-5-cyclo-hexapentanoyl-N-hexylamide, Sankyo Company, Ltd., Tokyo, Japan); SKF108566 (E-alpha-2-[2-butyl-1-(carboxy phenyl) methyl] 1H-imidazole-5-yl[methylane]-2-thiophenepropanoic acid, Smith Kline Beecham Pharmaceuticals, PA);
5 Losartan (DUP753/MK954, DuPont Merck Pharmaceutical Company); Remikirin (RO42-5892, F. Hoffman LaRoche AG); A₂ agonists (Marion Merrill Dow) and certain non-peptide heterocycles (G.D.Searle and Company).

"Angiotensin converting enzyme (ACE), is an enzyme which catalyzes the conversion
10 of angiotensin I to angiotensin II. ACE inhibitors include amino acids and derivatives thereof, peptides, including di- and tri- peptides and antibodies to ACE which intervene in the renin-angiotensin system by inhibiting the activity of ACE thereby reducing or eliminating the formation of pressor substance angiotensin II. ACE inhibitors have been used medically to treat hypertension, congestive heart failure, myocardial infarction and renal disease.
15 Classes of compounds known to be useful as ACE inhibitors include acylmercapto and mercaptoalkanoyl prolines such as captopril (US Patent Number 4,105,776) and zofenopril (US Patent Number 4,316,906), carboxyalkyl dipeptides such as enalapril (US Patent Number 4,374,829), lisinopril (US Patent Number 4,374,829), quinapril (US Patent Number 4,344,949), ramipril (US Patent Number 4,587,258), and perindopril (US Patent Number
20 4,508,729), carboxyalkyl dipeptide mimics such as cilazapril (US Patent Number 4,512,924) and benazapril (US Patent Number 4,410,520), phosphinylalkanoyl prolines such as fosinopril (US Patent Number 4,337,201) and trandolopril.

"Renin inhibitors" are compounds which interfere with the activity of renin. Renin inhibitors include amino acids and derivatives thereof, peptides and derivatives thereof, and
25 antibodies to renin. Examples of renin inhibitors that are the subject of United States patents are as follows: urea derivatives of peptides (US Patent Number 5,116,835); amino acids connected by nonpeptide bonds (US Patent Number 5,114,937); di- and tri- peptide derivatives (US Patent Number 5,106,835); amino acids and derivatives thereof (US Patent Numbers 5,104,869 and 5,095,119); diol sulfonamides and sulfinyls (US Patent Number
30 5,098,924); modified peptides (US Patent Number 5,095,006); peptidyl beta-aminoacyl aminodiols carbamates (US Patent Number 5,089,471); pyrrolimidazolones (US Patent Number 5,075,451); fluorine and chlorine statine or statone containing peptides (US Patent Number 5,066,643); peptidyl amino diols (US Patent Numbers 5,063,208 and 4,845,079); N-

morpholino derivatives (US Patent Number 5,055,466); pepstatin derivatives (US Patent Number 4,980,283); N-heterocyclic alcohols (US Patent Number 4,885,292); monoclonal antibodies to renin (US Patent Number 4,780,401); and a variety of other peptides and analogs thereof (US Patent Numbers 5,071,837, 5,064,965, 5,063,207, 5,036,054, 5,036,053, 5,034,512, and 4,894,437).

Agents that bind to cellular adhesion molecules and inhibit the ability of white blood cells to attach to such molecules include polypeptide agents. Such polypeptides include polyclonal and monoclonal antibodies, prepared according to conventional methodology. Such antibodies already are known in the art and include anti-ICAM 1 antibodies as well as other such antibodies (see earlier discussion on antibodies).

Other than aspirin, ticlopidine is another antiplatelet agent that has been shown to be beneficial for stroke treatment. Endarterectomy may be indicated in patients with 70 to 99 percent narrowing of a symptomatic internal carotid artery. However, most authorities agree that carotid endarterectomy is not indicated in patients with TIAs that are referable to the basilar-vertebral system, in patients with significant deficits from prior strokes, or in patients in whom a stroke is evolving.

HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) reductase is the microsomal enzyme that catalyzes the rate limiting reaction in cholesterol biosynthesis (HMG-CoA6Mevalonate). An HMG-CoA reductase inhibitor inhibits HMG-CoA reductase, and as a result inhibits the synthesis of cholesterol. A number of HMG-CoA reductase inhibitors has been used to treat individuals with hypercholesterolemia. More recently, HMG-CoA reductase inhibitors have been shown to be beneficial in the treatment of stroke (Endres M, et al., *Proc Natl Acad Sci U S A*, 1998, 95:8880-5).

HMG-CoA reductase inhibitors useful for co-administration with the agents of the invention include, but are not limited to, simvastatin (U.S. Patent No. 4, 444,784), lovastatin (U.S. Patent No. 4,231,938), pravastatin sodium (U.S. Patent No. 4,346,227), fluvastatin (U.S. Patent No. 4,739,073), atorvastatin (U.S. Patent No. 5,273,995), cerivastatin, and numerous others described in U.S. Patent No. 5,622,985, U.S. Patent No. 5,135,935, U.S. Patent No. 5,356,896, U.S. Patent No. 4,920,109, U.S. Patent No. 5,286,895, U.S. Patent No. 5,262,435, U.S. Patent No. 5,260,332, U.S. Patent No. 5,317,031, U.S. Patent No. 5,283,256, U.S. Patent No. 5,256,689, U.S. Patent No. 5,182,298, U.S. Patent No. 5,369,125, U.S. Patent No. 5,302,604, U.S. Patent No. 5,166,171, U.S. Patent No. 5,202,327, U.S. Patent No. 5,276,021, U.S. Patent No. 5,196,440, U.S. Patent No. 5,091,386, U.S. Patent No. 5,091,378, U.S. Patent

No. 4,904,646, U.S. Patent No. 5,385,932, U.S. Patent No. 5,250,435, U.S. Patent No. 5,132,312, U.S. Patent No. 5,130,306, U.S. Patent No. 5,116,870, U.S. Patent No. 5,112,857, U.S. Patent No. 5,102,911, U.S. Patent No. 5,098,931, U.S. Patent No. 5,081,136, U.S. Patent No. 5,025,000, U.S. Patent No. 5,021,453, U.S. Patent No. 5,017,716, U.S. Patent No. 5,001,144, U.S. Patent No. 5,001,128, U.S. Patent No. 4,997,837, U.S. Patent No. 4,996,234, U.S. Patent No. 4,994,494, U.S. Patent No. 4,992,429, U.S. Patent No. 4,970,231, U.S. Patent No. 4,968,693, U.S. Patent No. 4,963,538, U.S. Patent No. 4,957,940, U.S. Patent No. 4,950,675, U.S. Patent No. 4,946,864, U.S. Patent No. 4,946,860, U.S. Patent No. 4,940,800, U.S. Patent No. 4,940,727, U.S. Patent No. 4,939,143, U.S. Patent No. 4,929,620, U.S. Patent No. 4,923,861, U.S. Patent No. 4,906,657, U.S. Patent No. 4,906,624 and U.S. Patent No. 4,897,402, the disclosures of which patents are incorporated herein by reference.

Nitric oxide (NO) has been recognized as a messenger molecule with many physiologic roles, in the cardiovascular, neurologic and immune systems (Griffith, TM et al., *J Am Coll Cardiol*, 1988, 12:797-806). It mediates blood vessel relaxation, neurotransmission and pathogen suppression. NO is produced from the guanidino nitrogen of L-arginine by NO Synthase (Moncada, S and Higgs, EA, *Eur J Clin Invest*, 1991, 21:361-374). Agents that upregulate endothelial cell Nitric Oxide Synthase include, but are not limited to, L-arginine, *rho* GTPase function inhibitors (see International Application WO 99/47153, the disclosure of which is incorporated herein by reference), and agents that disrupt actin cytoskeletal organization (see International Application WO 00/03746, the disclosure of which is incorporated herein by reference).

"Co-administering," as used herein, refers to administering simultaneously two or more compounds of the invention (e.g., anagrelide, and an agent known to be beneficial in the treatment of, for example, a cardiovascular condition e.g., an anticoagulant-), as an admixture in a single composition, or sequentially, close enough in time so that the compounds may exert an additive or even synergistic effect, i.e., on reducing cardiomyocyte cell-death in a cardiovascular condition.

It should be understood that the preceding is merely a detailed description of certain preferred embodiments. It therefore should be apparent to those of ordinary skill in the art that various modifications and equivalents can be made without departing from the spirit and scope of the invention. It is intended that the invention encompass all such modifications within the scope of the appended claims.

All references, patents and patent applications and publications that are cited or referred to in this application are incorporated in their entirety herein by reference.

We claim: